EnzyChrom™ Nitric Oxide Synthase Inhibitor Screening Kit (EINO-100)

Quantitative Determination of Nitric Oxide Synthase Inhibitor Activity at 540 nm

DESCRIPTION

Nitric oxide (NO) is a reactive radical that plays an important role in many key physiological functions. NO is the oxidation product of arginine by nitric oxide synthase (NOS) and is involved in host defense development, activation of regulatory proteins and direct covalent interaction with functional biomolecules. Inhibition of NOS has the potential to produce diverse biological effects, particularly in the cardiovascular system.

Simple, direct and non-radioactive procedures for measuring NOS are becoming popular in research and drug discovery. BioAssay Systems' EnzyChrom™ Nitric Oxide Synthase Inhibitor Screening Kit involves two steps: a NOS reaction step during which NO is produced followed by an NO detection step. Since the NO generated by NOS is rapidly oxidized to nitrite and nitrate, the NO production is measured following reduction of nitrate to nitrite using an improved Griess method.

KEY FEATURES

High-throughput. Homogenous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling system.

Rapid and reliable. Can be completed in less than 3 hours if assay performed at 37°C.

APPLICATIONS

HTS for inhibitor screening and evaluation of NOS inhibitors.

KIT CONTENTS (100 tests in 96-well plates)

Assay Buffer:	10 mL	**Substrate**:	600 µL	**GDH**:	120 µL
Reagent A:	12 mL	**Reagent B**:	500 µL	**Reagent C**:	12 mL
Reagent D:	Dried	**Reagent E**:	1.5 mL		

Bulk Reagents: Custom sizes available upon request.

Storage conditions. The kit is shipped on ice. Store Reagent A, B, and C between -20 to 4°C. Store all other reagents at -20°C. Shelf life of six months after receipt. Use Reagent D within 1 week after reconstitution.

Precautions: reagents are for research use only. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

This assay is based on an enzyme-catalyzed kinetic reaction. To ensure identical incubation time, addition of Working Reagent should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. *Note: Neither the enzyme NOS nor a control inhibitor is included in the kit.*

Reagent Preparation: Prior to assay, equilibrate all components to room temperature. Reconstitute Reagent D with 300 µL dH_2O. Store unused reconstituted Reagent D at -20˚C and use within 1 week. Prewarm Assay Buffer to 25°C. Keep GDH on ice. If precipitates are present in Reagent B, warm at 37˚C until redissolved (~10-15 min). The Working Reagent should be prepared freshly and used within 30 min.

Sample Preparation: Dilute purified NOS to 12.5 U/mL using dH_2O or diluent. Dissolve the test compounds in solvent of choice. It is prudent to first test the tolerance of the solvent by the enzyme of choice. If using DMSO, the DMSO concentration should be 20 v/v% or less in the 5 µL of test compounds added to the reaction when screening with iNOS from mouse.

The following protocol is optimized for iNOS from mouse. If another species is being analyzed, we recommend that you experimentally determine the K_m and then adjust the volume of substrate in the Working reagent so that the final concentration of the substrate in the 50 µL reaction is near the K_m.

NOS Reaction Preparation:

1. Transfer 10 µL of NOS into separate wells.
2. Reserve at least one NOS well for no substrate (Blank), and one without inhibitor (Control).
3. To the Control and Blank well, add 5 µL of solvent that the test compounds are dissolved in. For example, if the test compounds are dissolved in 20 v/v% DMSO, add 5 µL 20 v/v% DMSO to these wells.
4. To the remainder of the wells containing NOS, add 5 µL of the test compounds.
5. Add 25 µL assay buffer to all wells and incubate the plate for 15 minutes at 25°C
6. Prepare sufficient Reaction Mix (RM) by mixing for each well (except Blank well), 2 µL Reagent D, 5 µL Reagent E, 5 µL Substrate, and 0.5 µL GDH. Prepare Blank Reaction Mix (BRM) by mixing for each blank well, 2 µL Reagent D, 5 µL Reagent E, 5 µL dH_2O and 0.5 µL GDH. Add 10 µL BRM to the Blank wells. Add 10 µL RM to the remaining wells. Tap plate to mix briefly and thoroughly. Incubate 60 minutes at 37°C.
7. After 60 min, prepare NO Detection Reagent (NO DR) by mixing per reaction well: 100 µL Reagent A, 4 µL Reagent B, and 100 µL Reagent C. Immediately add 200 µL of NO DR to each well. Run the detection reaction at 37°C for 60 min. Read OD at 500-570 nm (peak 540 nm).

CALCULATION

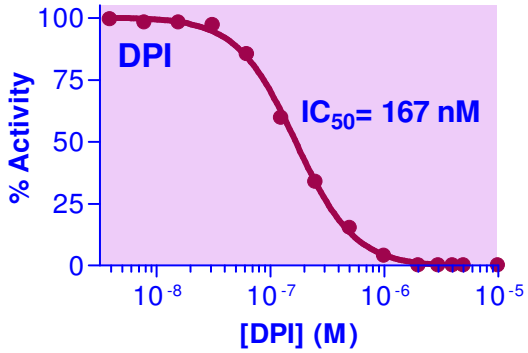
NOS inhibition for a test compound is calculated as follows:

$$\% \text{ Inhibition} = \left(1 - \frac{\Delta OD_{\text{Test Cpd}}}{\Delta OD_{\text{No Inhibitor}}}\right) \times 100\%$$

Where $\Delta OD_{\text{Test Cpd}}$ is the OD540 nm value of a test compound minus the OD 540 nm value of the Blank well (no substrate) at 60 min and $\Delta OD_{\text{No Inhibitor}}$ is the OD540 nm value of a no inhibitor (Control) minus the OD540 nm value of the Blank well (no substrate) at 60 min.

MATERIALS REQUIRED, BUT NOT PROVIDED

Purified NOS (e.g. Sigma Aldrich cat# N2783) and if desired a control NOS inhibitor (e.g. DPI, Sigma Aldrich Cat# D2926). Pipeting devices and accessories (e.g. multi-channel pipettor), clear flat bottom 96-well plates (e.g. VWR cat# 82050-760), and plate reader.



DPI titrations: iNOS from mouse was incubated with various concentrations of DPI. Each concentration of inhibitor contained 20 v/v% DMSO (final 2 v/v% in 50 µL reaction).

LITERATURE

1. Víteček, Jan, et al. (2012). Arginine-based inhibitors of nitric oxide synthase: therapeutic potential and challenges. Mediators of inflammation, 2012.
2. Mendes, A. F., et al. (2001). Diphenyleneiodonium inhibits NF-κB activation and iNOS expression induced by IL-1β: involvement of reactive oxygen species. Mediators of inflammation, 10(4), 209-215.
3. Boer, R., et al. (2000). The inhibitory potency and selectivity of arginine substrate site nitric-oxide synthase inhibitors is solely determined by their affinity toward the different isoenzymes. Molecular Pharmacology, 58(5), 1026-1034.